IMMUNOASSAY MONITORING for ATRAZINE in TEXAS

Alan Cherepon, Steve Musick, Joseph Peters

Texas Natural Resource Conservation Commission
Office of Environmental Policy, Analysis and Assessment, Water Quality Planning & Assessment Section
MC-147, P.O. Box 13087
Austin, Texas 78711-3087

ABSTRACT

The member agencies and entities of the Texas Groundwater Protection Committee, with the Texas Natural Resource Conservation Commission (TNRCC) serving as the lead agency, have prepared the state's Generic Pesticide Management Plan (PMP) for the prevention of pesticide contamination of groundwater. Texas received EPA concurrence for its PMP on June 6, 2000. TNRCC continues development and testing of the PMP's various components, especially groundwater monitoring.

Initially the most essential PMP component is ambient groundwater monitoring, since knowledge of areas and concentrations of pesticide contamination is prerequisite to carrying out many of the other components. Groundwater monitoring is performed in three ways. (1) Vulnerable area monitoring in areas found to be vulnerable to pesticide contamination. (2) Investigative monitoring for municipalities with pesticides detected in their public water supplies. (3) Cooperative monitoring to determine general ambient conditions.

Vulnerable area and Investigative monitoring is conducted by TNRCC personnel. The groundwater samples are analyzed using immunoassay techniques for atrazine and metolachlor. Samples are then selected for laboratory confirmation based on immunoassay results. Cooperative monitoring is carried out by the cooperating entity, who send samples to the TNRCC for immunoassay analysis. Wells with atrazine detections >0.3 ppb under cooperative monitoring will be resampled at a later date for verification. This "cut-off" concentration is one of several practical insights that are shared from Texas' immunoassay experience for pesticides.

Regions monitored include the High Plains aquifer in the Panhandle, the Gulf Coast aquifer and various aquifers in the Hill Country region of Central Texas. This paper presents monitoring results for atrazine in these regions.

BIOGRAPHICAL SKETCH

Alan Cherepon received his Bachelor of Art in Geology from Rutgers University, New Jersey, in 1976. He started employment with the Texas Natural Resource Conservation Commission in July of 1998, prior to which he has 20 years of hydrogeological and environmental experience, as well as 4 years of teaching secondary level science. He also holds a Texas Teacher Certification in secondary composite science. He is currently a Resource Conservation Specialist V and Sample Team Leader for the Groundwater Planning & Assessment Team of TNRCC, assisting his supervisor, Steve Musick, and Dr. Joseph Peters in FIFRA Grant/Pesticide Management Plan-related groundwater protection work.

1. INTRODUCTION

Immunoassay (IA) analysis methods began as early as 1959, initially in the medical field, and was initially recommended for use in the environmental field in 1971 (Reference 1). Test kits have been commercially available since about 1990, but widespread environmental field application of immunoassay technology has occurred only since the mid-1990's. Presently, about 12 commercial manufacturers provide these kits. EPA promulgated the IA method for atrazine and triazine analysis in 1999, as EPA Method 4670 (Reference 2).

Previous papers by TNRCC's Groundwater Planning & Assessment staff (Reference 3) addressed the practical aspects of their application of immunoassay analysis in Texas, and results from the Panhandle Cooperative Project (Reference 4). This paper will focus on atrazine monitoring results in the Texas Panhandle, as conducted under the FIFRA/Texas' Pesticide Management Plan (PMP) program.

Texas has aggressively sought to make their PMP both effective and efficient, as groundwater resources are irreplaceable, and agriculture is a major part of the State's overall economy. Texas has more acres in farm lands than any state in the U. S., and is second in agricultural production revenue **(**Reference 5). Atrazine is one of the most popular pesticides used for controlling weeds in corn, sorghum, and several other important crops. As analytical methods with increasingly lower detection levels are employed, an increasing number of detections are occurring in both surface water and groundwater in Texas.

The TNRCC is the State lead agency in the protection and regulation of groundwater resources in Texas. The TNRCC has the responsibility of chairing the Agricultural Chemicals Subcommittee and its parent Groundwater Protection Committee, the multi-agency bodies that direct and oversee protection of State groundwater resources from pesticide contamination. The Committee has responsibility for the development of the generic PMP and pesticide-specific PMPs, conducting groundwater monitoring in areas vulnerable to groundwater contamination by pesticides, and the investigation of areas with detects of pesticides in the groundwater. The monitoring and investigative activities for the fully-implemented PMP is anticipated to require considerable funding to cover the entire State.

One pilot site investigation in the Texas Panhandle expended nearly the entire annual sampling budget, leaving little funding for routine monitoring activities during the final quarter of the 1999 Fiscal Year. The IA method of pesticide analysis reduces the total number of costly lab analyses, which allows for increased sample coverage and a reliable and more efficient program. IAs are generally useful for the detection of triazine herbicides, especially atrazine. Method descriptions are provided in several references, most notably Reference#3. The ELISA IA method for atrazine is used by the TNRCC, and has proven to be useful for ambient monitoring, as well as vulnerable and high-use areas and contamination response. The small sample volume required for IA analysis has helped facilitate a recent surge in cooperative monitoring efforts between TNRCC, other state agencies, and groundwater management districts. The first of such efforts was conducted in the Panhandle region of Texas, where a significant percentage of crops to which atrazine is applied are grown. Since then, cooperative monitoring has continued in other regional aquifers in Texas. Results of the Texas atrazine monitoring program are presented below.

2. PROJECT STRUCTURE AND METHODOLOGY

a. Background

The Texas Generic Pesticide Management Plan (PMP) for Prevention of Pesticide Contamination of Groundwater, which was concurred by EPA in June 2000, specifies what groundwater protection programs are in place, detailing the potential scenarios requiring investigation. Included are two sampling scenarios; to indicate the source, source type, extent and magnitude of a pesticide impact, and to monitor areas vulnerable to pesticide contamination of groundwater, as well as high-use areas. A major aspect of the program is the Quality Assurance Project Plan (QAPP), which includes Standard Operating Procedures (SOP). The QAPP is the multi-part document that specifies groundwater sampling protocol and guidelines, acceptable analysis methods, and program standards. TNRCC's QAPP includes pesticide analysis by immunoassay method for screening and investigative (delineation) efforts.

b. Project Origin

The State Legislature established primary responsibility for the PMP program with the TNRCC as state lead agency, to be assisted by the members of the Agricultural Chemicals Subcommittee (ACS). The Texas Panhandle Region is the largest vulnerable area and highest atrazine use area in the state, having substantial amounts of corn and sorghum production, the main croplands on which atrazine is typically applied. In previous sampling results by various agencies, atrazine has been the primary pesticide to consistently show up in the region's groundwater. TNRCC's Groundwater Planning & Assessment Team (GPAT) has conducted several test investigations and vulnerable area monitoring activities in the region, utilizing immunoassay analyses to be more efficient and expedient in the process. Through communication and coordination with TNRCC's Public Drinking Water Section related to atrazine detects in several Public Water Supply (PWS) systems, the central Panhandle area was soon delineated as the primary focus for atrazine monitoring efforts in the state. These activities were reported to the ACS during quarterly meetings, caught the attention of the Texas Water Development Board (TWDB) Representative, who recognized an opportunity to promote the PMP vulnerable areas monitoring component. Sampling could be conducted during the TWDB's 5-year cycle of state groundwater quality monitoring, beginning with the Panhandle region in 1999. The High Plains Underground Water Conservation District #1 (HPUWCD#1), the largest of such organizations in the state, was also coordinating their routine water quality monitoring activities with the TWDB, and volunteered to provide additional samples, preferably in areas the TWDB had limited sample coverage. Since this early success, additional regional aquifers have been screened for both atrazine and metolachlor. These include the Gulf Coast, Hill Country, West Texas, Seymour/Blaine, and Nacatoch/Woodbine aquifers. These combined efforts, coordinated through the ACS, enabled the efficient ambient screening of regional aquifers for atrazine and metolachlor.

c. Project Objectives and Structure

The Panhandle Cooperative Project's (PCP) primary objective was to screen groundwater samples for atrazine from the Texas High Plains aquifer. Wells with atrazine concentrations at or greater than 0.3 ppb would be recommended for follow-up verification sampling, as TNRCC's experience indicates samples with concentrations below this will come back as non-detects by the more costly lab analyses. This approach would address the PMP monitoring component and the fostering of cooperation of state and local agencies and entities.

Project sampling was primarily conducted by the TWDB, with additional sampling by the HPUWCD #1. Field notations and well records were the responsibility of the sampling entity, while analysis, compilation of the data, map and report generation were TNRCC's responsibilities. All immunoassay analyses for atrazine were conducted by TNRCC's GPAT staff (in the Panhandle by Alan Cherepon & Joseph Peters; other regional aquifers by Alan Cherepon & Abiy Berehe).

Since TNRCC's activities and related expenses were conducted under the FIFRA Grant, the program's QAPP was required. This allows for other entities to conduct sampling for the program, as long as they are trained to follow sampling and shipping protocol. Both TWDB and HPUWCD#1 sampling personnel underwent TNRCC training to fulfill grant requirements, to ensure adequate QA/QC measures were maintained. These included;

- collecting 1 duplicate sample for every 20 samples,
- 1 field blank for every 20 samples,
- Collection of sample in TNRCC-supplied 40-ml glass vials, leaving no headspace (bubbles),
- keeping samples cooled from 2^0-4^0Celsius, in a secured place until delivered to TNRCC for immunoassay analysis, and accompanied by a chain-of-custody

Sampling was conducted by field staff who would work separately on individual counties or areas each week. TWDB staff would check with the HPUWCD #1 for their samples, and then deliver all samples for the week at TNRCC for analysis. All vials included labels, and Chains-of-Custody were provided on all samples,

documenting sampler, county, well ID, time, date, how transported, when released to TNRCC, and whether sample preservation temperature of 2^0-4^0 C was maintained.

Actual analysis of samples were conducted by TNRCC's GPAT staff at the Austin headquarters, building B Lab. Several commercially available IA systems exist, with TNRCC staff using the SDI/Ohmicron kits and instrument for magnetic-particle-based **e**nzyme-**l**inked **i**mmuno**s**orbant **a**ssay (ELISA) method of analysis of atrazine in water samples. Analysis was done using two SDI/Ohmicron RaPID Analyzer spectrophotometers. The RaPID Assay kits for atrazine have a Least Detectable Dose (LDD, which is similar to the method reporting limits) of 0.05 ppb and 5.0 ppb, with the optimal range for the method being in the 0.3 to 1.0 ppb area (most accurate and precise values are in this range). "False positive" results are typically attributed to the method detecting parent compound, structurally related compounds (triazines for atrazine), and metabolites all as the parent compound, which is why lab analysis concentrations are usually less than the IA concentrations. Texas' results typically indicate roughly twice as high atrazine concentration by IA analysis as by laboratory GC/MS method. Most samples indicating concentrations of pesticides >0.3 ppb by the IA method are recommended for verification by lab analysis, while those <0.3 ppb have resulted in non-detection by lab analysis and are not typically sent for lab analysis.

Additionally, TNRCC's staff were responsible for analytical data compilation, GIS mapping of results, and both update and final reports on the project. The TWDB and HPUWCD #1 were each responsible for sample and well data records, making these available to TNRCC staff as needed. Following completion of the High Plains, the Gulf Coast, Hill Country, West Texas, Seymour/Blaine, and Nacatoch/Woodbine aquifers were sampled during the 2001 fiscal year. Metolachlor was added to the immunoassay analysis.

3. DATA

The data used in this paper was accumulated between March 2000 and October 2001, requiring approximately 8 months to screen a good portion of the High Plains aquifer. Analytical data was entered regularly into a Paradox© database for final review and use within the ArcView© GIS software for map construction (Figures 1-3). Upon completion of the analyses, the database totals were compared to Chains-of-Custody and immunoassay analysis sheets to determine actual totals, numbers of QA/QC samples, and how many samples were collected by each group. Several wells that were missing latitude/longitude data were further researched, with all but one well accounted for (possibly being an unmarked duplicate sample). The sampling is summarized in the tables below, followed by the data QA/QC summary.

High Plains Aquifer Sample Summary Table

Agency/Entity	# of Wells Sampled	# QA/QC Samples
TWDB	634	
HPUWCD#1	87	
TOTALS	721	61

Table 1 - 2001 Cooperative Ambient Groundwater Monitoring of Pesticides in Texas

Aquifer Name	# of Counties	# of Wells Sampled	# of Samples Analyzed
Gulf Coast	47	391	414
Hill Country	11	38	42
Seymour/Blaine	6	13	16
West Texas Bolson	3	25	28
Woodbine/Nacatoch	9	20	23
Totals	76	449	523

Immunoassay data sheets were maintained for each analytical run, documenting which staff did the analysis, date and time analyzed, instrument number, calibration data, sample ID, concentration, date and time of sample collection, and other important notes.

· RESULTS

From 2000 through 2001, nearly 1100 well samples were analyzed for atrazine by IA method. This essentially covered most of the High Plains aquifer in the Texas Panhandle, the Gulf Coast aquifer along the coast, the Hill Country aquifer in the center of the state, the West Texas aquifers in far west Texas, the Seymour and Blaine aquifers east of the High Plains, and the Nacatoch and Woodbine aquifers in East Texas. Sampling and analysis were completed within about 16 months of a two year period, with about 3-4 months additional time for data reduction, analysis, mapping, and reporting of results. The attached maps (Figures 1&2) provides a view of the aquifer regions. The following bulleted and tabulated summary further details project findings, first, in the High Plains, followed by the other aquifers:

High Plains Aquifer Results

- 206 well samples had atrazine detects
- 515 well samples did not have atrazine detected (ND = not detected)
- 5 well samples had atrazine detects greater than 1.0 ppb
- 21 samples indicated atrazine concentrations between 1.0 and 0.3 ppb
- 26 wells recommended for re-sampling/verification by laboratory analysis
- 6 counties had more than one detect above 0.3 ppb atrazine, centering around Castro County
- 4 additional counties had one detect above 0.3 ppb
- Results for 172 samples are considered close approximations due to minor QA/QC problems with one calibration factor during immunoassay analysis

Summary of Atrazine Analyses Results for the High Plains Aquifer

Total # of Wells Analyzed	# of Non-Detects (ND is <0.05ppb)	# of Detects ≥0.05<0.1ppb	# of Detects ≥0.1<0.3ppb	# of Detects ≥0.3<1.0ppb	# of Detects ≥1.0ppb
721	515	117	63	21	5

The most notable trend is the cluster of detects stretching from Deaf Smith County to Hale County, in the central area of the Panhandle region. These results correspond with a pattern of Public Water Supply systems (Figure 3) having relatively high atrazine detects in specific wells, which have been undergoing investigation by

the Agricultural Chemicals Subcommittee of the Texas Groundwater Protection Committee.

Table 2 - Analytical Results, 2001 Ambient Groundwater Monitoring of Pesticides in Texas

Aquifer Name	# of Detects < 0.05 (ND) Atr./Met.	# of Detects > 0.05 < 0.1 Atr./Met.	# of Detects > 0.1 < 0.3 Atr./Met.	# of Detects >0.3 < 1.0 Atr./Met.	Highest Concentration Atr./Met.
Gulf Coast	406/404	4/6	1/4	3/0	0.47/0.18
Hill Country	36/41	3/0	3/0	0/1	0.18/0.64
Seymour/Blaine	16/16	0/0	0/0	0/0	0/0
West Texas	28/28	0/0	0/0	0/0	0/0
Woodbine/Nacatoch	23/23	0/0	0/0	0/0	0/0
Totals	442/445	7/6*	4/4*	3/1*	

*4 Blanks had atrazine detects, and 1 blank had a metolachlor detect

13. CONCLUSIONS

Atrazine has been the only PMP-herbicide that has consistently and overwhelmingly been detected in the High Plains aquifer of the Texas Panhandle Region. This, along with budgetary limitations, resulted in atrazine being the only chemical analyzed for in High Plains aquifer. Several factors caused the addition of metolachlor in the screening of the other aquifers in 2001. The results have proved this to be a prudent decision. This cooperative project indicates a pattern (Figure 3) of atrazine detects in the central area of the Texas Panhandle. Prior sample results from monitoring of PWS systems has resulted in a similar pattern in PWS systems with atrazine detects. Such region-wide groundwater monitoring further supports earlier indications in the PWS systems that a regional groundwater contamination concern exists for atrazine in the Texas Panhandle. This will enable educational and Best Management Practice efforts to be conducted regionally, rather than piecemeal. The end result should be a state-wide savings of funds and resources, as well as speeding up actions to reverse this atrazine contamination trend, and hopefully will improve groundwater quality in the region before it becomes a more serious and costly health concern. The cost estimate comparison further confirmed the programs efficiency;

- Non-Cooperative Sampling & Laboratory Estimated Cost
  1. ~50 Man-Weeks of sampling staff salaries ($27,000) plus expenses ($17,000)
  2. 782 total samples X ~$240/Method 525 analysis = ~$190,000
  3. Total Sampling & Analytical Cost $230,000
- Cooperative Project Immunoassay and Follow-up Estimated Cost
  1. 782 samples or 10 reagent kits + pipette tips X $430 = $4,300 + $5,700 test equipment
  2. TNRCC Staff Time ~160 Man-Hours X $770/40 = $3077
  3. Total Analytical Cost $13,077
- **Total Savings to State = $216,923**

The cooperative atrazine groundwater monitoring by immunoassay method as conducted by the TWDB, HPUWCD#1, and TNRCC enabled a large area of the state to be screened for potential atrazine contamination. The Panhandle Region is primarily underlain by the High Plains/Ogallala Aquifer. This is also the largest vulnerable area for atrazine contamination of

groundwater, and the highest atrazine use area of the state. Such monitoring of vulnerable areas is a required component of the Texas Generic Pesticide Management Plan. Conducting the monitoring by coordination between state agencies and other water resource entities has enabled this work to be conducted quickly and efficiently, saving the state considerable resources, while speeding up groundwater protection activities. The State of Texas is coordinating continued cooperative groundwater monitoring during 2002 in the Carrizo-Wilcox aquifers, which provides a substantial savings to taxpayers, and is accomplished in a more timely manner.

· RECOMMENDATIONS

The following recommendations are based on project results, and the authors' experiences with Immunoassay for atrazine:

- Continue to coordinate IA screening with other agency subdivisions, agencies, and local entities, to share in related efforts and expenses and eventually cover the entire state; One group may not be able to afford the equipment or use it enough to justify the purchase, but several can share in the expenses and benefits
- Expand program, secure funding to screen for additional pesticides
- Spin-off project to determine source(s) of contamination and migration pathways in both playa and non-playa lake areas
- Secure funding to conduct sampling of well fields for larger cities, where entry points are only sampled and individual wells are seldom or never sampled and analyzed for pesticides

11. REFERENCES

1) Aga, D.S., and Thurman, E.M., Environmental Immunoassay: Alternate Technology for Soil and Water Analysis, 1997. American Chemical Society Symposium Series # 657, Chapter 1, Pp. 1- 20.

2) USEPA Method 4670, SW-846, Chapter 4.4, or 540/R94-509, 1999.

3) Musick, S., Peters, J., Cherepon, A., Immunoassay Analysis for the Determination of Pesticides in Groundwater Samples - The Texas Experience, National Water Quality Monitoring Conference, Austin, Texas, April, 2000, Proceedings, Pp. 317-327.

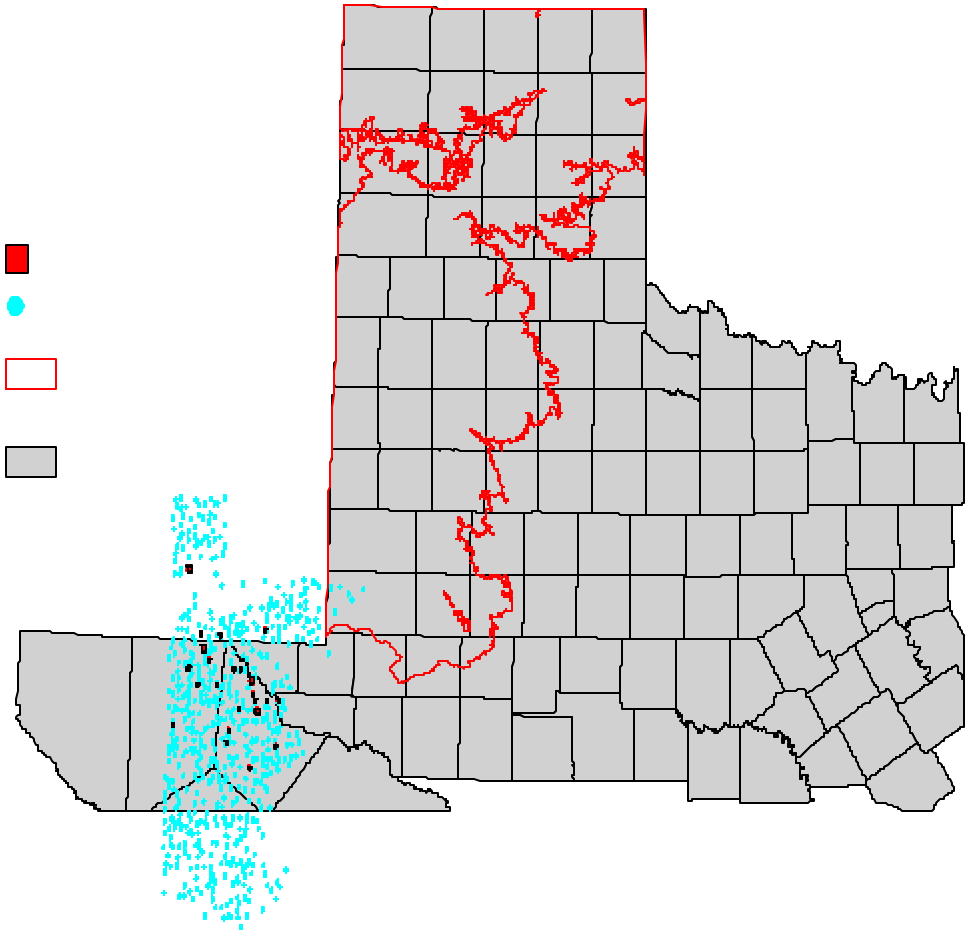
4) Cherepon, A., Musick, S., Peters, J., Atrazine Monitoring of the High Plains Aquifer by Immunoassay, Ground Water Protection Council Annual Forum, Reno, Nevada, September 22- 26, 2001, Proceedings, Pp.180-190.

5) USDA/TDA, 1997. Texas Agricultural Statistics Service, 1997.

Figure 1

Panhandle/High Plains Aquifer

Cooperative Well Sample Locations



Atrazine Detect >0.3ppb

Well Sample Location

High Plains Aquifer

Counties

Figure 2
2001 Cooperative Regional Aquifer
Monitoring Program

Figure 4

2001 Cooperative Ambient Groundwater Monitoring Locations

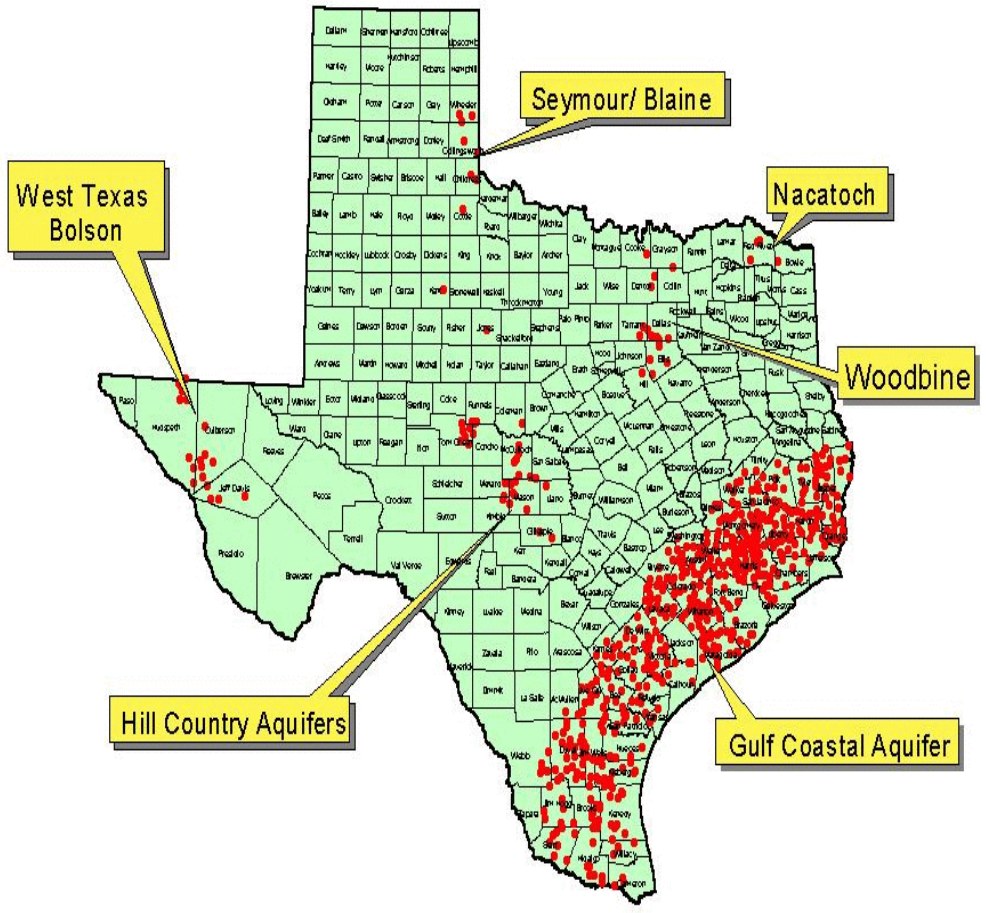


Figure 3
Wells with Atrazine Detects >0.3 ppb and
PWS with Atrazine Detects
(excluding city of Amarillo, no Atrazine detects to date)

Figure 5

Atrazine Detects in the Texas Panhandle